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(54) Title: USE OF CPG NUCLEIC ACIDS IN PRION-DISEASE

(57) Abstract: Methods are provided which are useful in the treatment of prion diseases and other protein deposit diseases, including, for example, post-exposure prophylaxis against the development of iatrogenic Creutzfeldt-Jakob disease. The methods involve the use of immunostimulatory nucleic acids, including CpG nucleic acids.

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USE OF CpG NUCLEIC ACIDS IN PRION DISEASE

Field of the Invention

The instant invention pertains to methods useful in the treatment of prion diseases, including, for example, post-exposure prophylaxis against the development of iatrogenic Creutzfeldt-Jakob disease. The methods involve the use of immunostimulatory nucleic acids.

Background of the Invention

Prion diseases include a number of fatal, neurodegenerative diseases believed to be caused by aggregates of normal protein that is present in an abnormal conformation. The normal protein, prion protein, is usually present in the cell membrane of many tissues, particularly neuronal tissue. The abnormally conformed prion protein is believed to be directly involved in converting normally conformed prion protein into more of the abnormally conformed prion protein, which then self-assembles into aggregates that are damaging to neuronal tissue anatomy and function.

At least some of the prion diseases are transmissible. However, unlike bacteria, viruses, fungi, parasites, and other replicating pathogens, transmissible prions are simply proteins; they are transmissible without any accompanying nucleic acid. For reasons that are not yet fully understood, the abnormally conformed prion proteins normally do not induce an immune response. Thus, exposure of a healthy individual to abnormally conformed prion protein can initiate a prion disease that can go unchecked by the immune system.

Exposure to abnormally conformed prion protein thus represents a health risk to susceptible individuals. Such individuals include humans and non-humans, principally cows and sheep. Exposure can come about through contact with prion-diseased animal products through ingestion, iatrogenic or work-related exposure, transplantation, and administration of pharmaceutical preparations.

Summary of the Invention

The instant invention is based in part on the unexpected discovery by the inventors that administration of immunostimulatory nucleic acid to a subject that is exposed to abnormally conformed prion protein is an effective treatment of prion disease. The immunostimulatory nucleic acid effectively delayed and even prevented disease in animals

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administered very large doses of prion-diseased brain homogenates that uniformly caused fatal disease in all untreated control animals similarly exposed to prion-diseased brain homogenates.

The immunostimulatory nucleic acids are useful in the treatment of prion diseases, including Creutzfeldt-Jakob disease (CJD), bovine spongiform encephalopathy (BSE), and scrapie. The methods of the invention are also useful for the study of these diseases, for instance, in animal models. The methods will also be useful for developing an understanding of how prion proteins normally fail to elicit an immune response and how an immune response can be elicited and used to treat prion protein disease.

The immunostimulatory nucleic acids are also useful in the treatment of other neurologic diseases involving abnormal protein deposits or aggregates. Such diseases include Alzheimer's disease, which involves deposits of amyloid. The main component of amyloid plaques is amyloid- β peptide (A β), a fibrillar 40-42 amino acid peptide that accumulates extracellularly and causes neuronal death.

In one aspect the invention provides a method for treating a prion disease in a subject. The method involves administering to a subject having or at risk of developing a prion disease a CpG nucleic acid in an effective amount to treat the prion disease. In one embodiment the administering follows exposure of the subject to a prion protein that is associated with a prion disease. In one embodiment the prion disease is a transmissible spongiform encephalopathy (TSE). In one embodiment the subject is a human.

In various preferred embodiments, the prion disease is scrapie, BSE, or a form of CJD. The CJD in one embodiment is iatrogenic CJD (iCJD). In another embodiment the CJD is variant CJD (vCJD). In yet another embodiment the CJD is sporadic CJD.

In one aspect the invention provides a method for inducing an immune response to a prion protein. The method according to this aspect of the invention involves the steps of contacting an antigen-presenting cell (APC) with a prion protein and contacting the APC with a CpG nucleic acid in an effective amount to induce an immune response to the prion protein. In one embodiment the immune response occurs in vivo. In one embodiment the immune response occurs in vitro. In all embodiments according to this aspect of the invention the APC is preferably chosen from a B cell, a dendritic cell, a macrophage, and a monocyte. In one preferred embodiment the APC is a dendritic cell.

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Also according to this aspect of the invention, in one embodiment the APC expresses a Toll-like receptor (TLR) that signals in response to the CpG nucleic acid. It has recently been reported that CpG nucleic acid can specifically induce a particular TLR, designated TLR9, to signal. Accordingly, in one embodiment the TLR is TLR9.

In one embodiment the prion protein includes prion protein:scrapie form (PrP^{Sc}). In another embodiment the prion protein includes a fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc} . In yet another embodiment the prion protein includes a derivative of PrP^{Sc} or a derivative of a fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc} .

In one embodiment the prion protein is prion protein:scrapie form (PrP^{Sc}). In another embodiment the prion protein is a fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc} . In yet another embodiment the prion protein is a derivative of PrP^{Sc} or a derivative of a fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc} .

It has been reported that certain CpG nucleic acids are more effective in one species than in another. Accordingly, in preferred embodiments the CpG nucleic acid is optimized for use in a species of the subject.

It has also been reported that CpG nucleic acids appear to fall into different classes based on certain structural features as well as their function. At least three classes are believed to exist, denoted Class A, Class B, and Class C. In one embodiment the CpG nucleic acid is a class B CpG nucleic acid. In one embodiment the CpG nucleic acid is a class A CpG nucleic acid. In one embodiment the CpG nucleic acid is a class C CpG nucleic acid.

These and other features of the invention are described in greater detail in connection with the detailed description of the invention.

Detailed Description of the Invention

A major step in the study of prions and the diseases that they cause was the discovery and purification of a protein designated prion protein (PrP). Bolton et al. (1982) *Science* 218:1309-11; Prusiner SB et al. (1982) *Biochemistry* 21:6942-50; McKinley MP et al. (1983) *Cell* 35:57-62. Complete prion protein-encoding genes have since been cloned, sequenced and expressed in transgenic animals. PrP^{C} is encoded by a single-copy host gene (Basler K et

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al. (1986) *Cell* 46:417-28) and is normally found at the outer surface of neurons. Prion diseases are accompanied by the conversion of PrP^C into a modified form called PrP^{Sc}.

The scrapie isoform of the prion protein (PrP^{Sc}) is thought necessary for both the transmission and pathogenesis of the transmissible neurodegenerative diseases of animals and humans. See Prusiner SB (1991) *Science* 252:1515-22. The most common prion diseases of animals are scrapie of sheep and goats and bovine spongiform encephalopathy (BSE; "mad cow disease") of cattle. Wilesmith J et al. (1991) *Microbiol Immunol* 172:21-38. Four prion diseases of humans have been identified: (1) kuru, (2) Creutzfeldt-Jakob disease (CJD), (3) Gerstmann-Sträussler-Scheinker (GSS) syndrome, and (4) fatal familial insomnia. Gajdusek DC (1977) *Science* 197:943-60; Medori et al. (1992) *N Engl J Med* 326:444-9.

Most CJD cases are sporadic, but about 10-15% are inherited as autosomal dominant disorders that are caused by mutations in the human PrP gene. Hsiao et al. (1990) *Neurology* 40:1820-7; Goldfarb et al. (1992) *Science* 258:806-8. Iatrogenic CJD has been caused by human growth hormone derived from cadaveric pituitaries as well as dura mater grafts. Brown et al. (1992) *Lancet* 340:24-7. Despite numerous attempts to link CJD to an infectious source such as the consumption of meat from scrapie-infected sheep, none has been identified to date (Harries-Jones et al. (1988) *J Neurol Neurosurg Psychiatry* 51:1113-9) except in cases of iatrogenically induced disease. On the other hand, kuru, which for many decades devastated the Fore and neighboring tribes of the New Guinea highlands, is believed to have been spread by infection during ritualistic cannibalism.

The major component of purified infectious prions, designated PrP 27-30, is the proteinase K resistant core of a larger native protein PrP^{Sc} which is the disease causing form of the ubiquitous cellular protein PrP^C. PrP^{Sc} is found only in scrapie infected cells, whereas PrP^C is present in both infected and uninfected cells implicating PrP^{Sc} as the major, if not the sole, component of infectious prion particles. Since both PrP^C and PrP^{Sc} are encoded by the same single copy gene, great effort has been directed toward unraveling the mechanism by which PrP^{Sc} is derived from PrP^C. Central to this goal has been the characterization of physical and chemical differences between these two molecules. Properties distinguishing PrP^{Sc} from PrP^C include low solubility (Meyer RK et al. (1986) *Proc Natl Acad Sci USA* 83:2310-4), poor antigenicity (Kascak RJ et al. (1987) *J Virol* 61:3688-93; Serban D. et al. (1990) *Neurology* 40:110-7), protease resistance (Oesch B et al. (1985) *Cell* 40:735-46), and polymerization of PrP 27-30 into rod-shaped aggregates which are very similar, on the

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ultrastructural and histochemical levels, to the PrP amyloid plaques seen in scrapie-diseased brains (Prusiner SB et al. (1983) *Cell* 35(2 Pt 1):349-58). By using proteinase K it is possible to denature PrP^C but not PrP^{Sc}. To date, attempts to identify any post-translational chemical modifications in PrP^C that lead to its conversion to PrP^{Sc} have proven fruitless. Stahl N et al. (1993) *Biochemistry* 31:5043-53. Consequently, it has been proposed that PrP^C and PrP^{Sc} are in fact conformational isomers of the same molecule.

Conformational description of PrP using conventional techniques has been hindered by problems of solubility and the difficulty in producing sufficient quantities of pure protein. However, PrP^C and PrP^{Sc} are conformationally distinct. Theoretical calculations based upon the amino acid sequences of PrPs from several species have predicted four putative helical motifs in the molecule. Experimental spectroscopic data would indicate that in PrP^C these regions adopt alpha-helical arrangements, with virtually no beta-sheet. Pan et al. (1993) *Proc Natl Acad Sci USA* 90:10962-6). In dramatic contrast, in the same study it was found that PrP^{Sc} and PrP 27-30 possess significant beta-sheet content, which is typical of amyloid proteins. Moreover, studies with extended synthetic peptides, corresponding to PrP amino acid residues 90-145, have demonstrated that these truncated molecules may be converted to either alpha-helical or beta-sheet structures by altering their solution conditions. The transition of PrP^C to PrP^{Sc} requires the adoption of beta-sheet structure by regions that were previously alpha-helical.

In general, scrapie infection fails to produce an immune response, with host organisms being tolerant to PrP^{Sc} from the same species. Polyclonal anti-PrP antibodies have been raised in rabbits following immunization with large amounts of Syrian hamster PrP 27-30. Bendheim PE et al. (1985) *Proc Natl Acad Sci USA* 82:997-1001; Bode L et al. (1985) *J Gen Virol* 66:2471-8. Similarly, a handful of anti-PrP monoclonal antibodies have been produced in mice. Kacsak RJ et al. (1987) *J Virol* 61:3688-93; Barry RA et al. (1986) *J Infect Dis* 154:518-21. These antibodies are able to recognize native PrP^C and denatured PrP^{Sc} from both Syrian hamsters and humans equally well, but do not bind to murine PrP. Unsurprisingly, the epitopes of these antibodies were mapped to regions of sequence containing amino acid differences between Syrian hamster and murine PrP. Rogers et al. (1991) *J Immunol* 147:3568-74.

The DNA sequence of the human, sheep and cow PrP genes have been determined, allowing, in each case, the prediction of the complete amino acid sequence of their respective

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PrP proteins. The normal amino acid sequence which occurs in the vast majority of individuals is referred to as the wild-type PrP sequence. This wild-type sequence is subject to certain characteristic polymorphic variations. In the case of human PrP, two polymorphic amino acids occur at residues 129 (Met/Val) and 219 (Glu/Lys). Sheep PrP has two amino acid polymorphisms at residues 136 and 171, while bovine PrP has either five or six repeats of an eight amino acid motif sequence (octarepeats) in the amino terminal region of the mature prion protein. While none of these polymorphisms are of themselves pathogenic, they appear to influence prion diseases. Distinct from these normal variations of the wild-type PrP proteins, certain mutations of the human PrP gene which alter either specific amino acid residues of PrP or the number of octarepeats have been identified which segregate with inherited human prion diseases.

For example, sequences of chicken, bovine, sheep, rat, and mouse PrP genes are disclosed and published within Gabriel JM et al. (1992) *Proc Natl Acad Sci USA* 89:9097-101. A sequence for a PrP gene of Syrian hamster is published in Basler K et al. (1986) *Cell* 46:417-28. A PrP gene of sheep is published by Goldmann W et al. (1990) *Proc Natl Acad Sci USA* 87:2476-80. A gene sequence for bovine PrP is published in Goldmann W et al. (1991) *J Gen Virol* 72:201-4. A sequence for chicken PrP gene is published in Harris DA et al. (1991) *Proc Natl Acad Sci USA* 88:7664-8. A PrP gene sequence for mink is published in Kretzschmar HA et al. (1992) *J Gen Virol* 73:2757-61. A human PrP gene sequence is published in Kretzschmar HA et al. (1986) *DNA* 5:315-24. A PrP gene sequence for mouse is published in Locht C et al. (1986) *Proc Natl Acad Sci USA* 83:6372-6. A PrP gene sequence for sheep is published in Westaway D et al. (1994) *Genes Dev* 8:959-69. These publications are all incorporated herein by reference to disclose and describe the PrP gene and PrP amino acid sequences.

Human PrP cDNA (SEQ ID NO:1; GenBank Accession No. M13899)

cggcgccgcg	agtttctctt	ctcctcacga	ccgaggcaga	gcagtcatta	tggcgaacct	60
tggctgctgg	atgctggttc	tctttgtggc	cacatggagt	gacctggccc	tctgcaagaa	120
gcgcggcaag	cctggaggat	ggaacactgg	gggcagccga	tacccggggc	agggcagccc	180
tggaggcaac	cgctaccac	ctcaggggcgg	tggtggtctgg	gggcagcctc	atggtgttgg	240
ctgggggcag	cctcatggtg	gtggctgggg	gcagccccat	ggtggtggtct	ggggacagcc	300
tcatggtgt	ggctgggttc	aaggaggtgg	cacccacagt	cagtggaaaca	agccgagtaa	360
gccaaaaacc	aacatgaagc	acatggctgg	tgctgcagca	gctggggcag	tggtgggggg	420
ccttggcggc	tacatgctgg	gaagtggcat	gagcaggccc	atcatacatt	tcggcagtga	480
ctatgaggac	cgttactatc	gtgaaaacat	gcaccgttac	cccaaccaag	tgtactacag	540
ccccatggat	gagtacagca	accagaacaa	ctttgtgcac	gactgcgtca	atatcacaat	600
caagcagcac	acggtcacca	caaccaccaa	gggggagaac	ttcaccgaga	ccgacgttaa	660
gatgatggag	cgcgtggtg	agcagatgtg	tatcacccag	tacgagaggg	aatctcaggc	720

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ctattaccag	agaggatcga	gcatggtcct	cttctccctc	ccacctgtga	tcctcctgat	780
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taatctttt	ccagcttgag	ggaggcggt	tccacctgca	gccctttag	tgggtgtgc	900
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actggaaaac	atagagtata	cctgagatgc	tggtaagcc	ccctttgatt	gagttcatca	1020
tgagccgtt	ctaatgccag	gccagtaaaa	gtataacagc	aaataaccat	tggtaatct	1080
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aaatacctt	gcctggatac	ctctggctcc	ttcagcagct	agagctcagt	atactaata	1200
cctatcttag	tagagattt	atagctattt	agagatattt	tccattttaa	gaaaacccga	1260
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aactgggtg	atgtttact	tttcacagta	tgggctacac	agcagctgtt	caacaagagt	1440
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aacatata	aaaggcttct	gggacttgaa	atcaaatgtt	tgggaatgtt	gcccttggag	1560
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caccatcatc	atagaggatg	atgttaattaa	aaaatggtc	gtgtgcaag	aaaagaactg	1800
cttgcattt	tttatttctg	tctcataatt	gtcaaaaacc	agaatttagt	caagttcata	1860
gttctgtaa	ttggctttt	aatcaaagaa	tagggagaca	atctaaaaaa	tatcttaggt	1920
tggagatgac	agaaatatga	ttgatttga	gtggaaaaag	aaattctgtt	aatgttaatt	1980
aaagtaaaat	tattccctga	attgtttgt	attgtcacct	agcagatatg	tattactttt	2040
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gtgtccctt	ggcttacaat	gtgcactgaa	tgcgttcatg	taagaatcca	aagtggacac	2280
cattaacagg	tcttgaaat	atgcatgtac	tttataatttt	ctatatttgc	aactttgcac	2340
gttcttgttt	tgttataataa	aaaaattgt	aatgttaat	atctgactg	aattaaacga	2400
gcgaagatga	gcacc					2415

Mink PrP genomic DNA (SEQ ID NO:2; GenBank Accession No. S46825)

tcattttgtt	ttgtttgtt	ttgtttgcag	ataagccatc	atggtaaaaa	gccacatagg	60
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gccaaaggct	ggaggaggct	ggaacactgg	ggggagccga	tacccaggcgc	agggcagtc	180
tggaggcaac	cgctacccac	cccagggtgg	ttggcgctgg	ggccagcccc	acgggggtgg	240
ctggggacag	ccccacgggg	ttggctgggg	tcagccccac	gggggtggct	ggggacagcc	300
gcatggtgc	ggtggctggg	gtcaaggtgg	ttggagccac	ggtcagttgg	gcaagcccag	360
taagccaaa	accaacatga	agcatgtggc	gggagccgca	gcagccgggg	cggtcgtgg	420
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caagccgg	gatcagtaca	gcaaccagaa	caacttcgt	catgactg	tcAACATCAC	600
ggtaagcag	cacacgggt	ccaccaccac	caagggcgcag	aacttcacgg	agaccgacat	660
gaagatcatg	gagcgcgtgg	ttggagcagat	gtgtgtcacc	cagtaccagc	gagagtccga	720
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tcttcac	ttacaggtt	ggggaggggg	tgtctaccta	cagccctgt	gtgggtgtgt	900
ctcattctt	tttgccttta	tcacccatag	gtaatcccc	ttggccctg	tggccctggg	960
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tttcaaaatc	tttgccttgg	aaacccctgg	tttgcctgtc	agctagagct	cagtacatta	1200
atgtccatc	ttagccgtt	tttgcctgtc	acttggggaa	tttttctcc	ccactctaaa	1260
agaacgcgt	tgcacttcc	tgtgcataa	acatttctgc	caaatttgc	aggaggccac	1320
atgatattca	ttcaaaaatc	aaaactagaa	accctttgc	tttgcacgc	agcccccgt	1380
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gaatgaacat	aatcaacagt	gcgaaaatgc	tacaaaaat	cccacactgg	ggagcagtgt	1560
ccttggaggc	aatttttttt	cctttggga	catttaaagc	ccctatatgt	ggcattccct	1620

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aaaagaaaata	ccttaggtca	cccacatgac	aaataaccat	tcaggtt	aaaaaggaat	1980
tctgttaact	gttatttaag	taaggcaaaa	ttattgtccg	gattgtcga	tatcatcagc	2040
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aaaaaaaaatt	attatatata	tttgttatga	caaactttaga	agttttgtct	agaggagtt	2160
acatctgata	tatctaattgc	accaccatgtt	ttggaaaggta	ctaaataactt	aatatgtaga	2220
aatccttttg	cgtggcctc	aggcttacac	gtgcactgaa	tagtttgt	tgatagagcc	2280
catgtggct	tcgaaatatg	catgtacttt	atattttcta	tattttaac	tggcatgt	2340
cttgataaaa	aaatgtataa	acattcgaac	tcttgactag	aattaaacag	gaactgagtg	2400
tgtcccatgt	gtttgcagt	acattcacca	ccgcaccctg	tgttgg		2446

Syrian Hamster PrP cDNA (SEQ ID NO:3; GenBank Accession No. M14054)

tcgaaaatct	cccttttag	caatttcttgc	ctccttagat	ttcagcaatt	gctttctcgc	60
tccattaggc	aacctttcat	tttctcacct	tccccattat	gtacgggag	caatgggttc	120
tggaccagtc	ttccattaaa	gatgatTTT	atagtcgggt	agcgcgtca	gggagtgtatg	180
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agcagcagac	cgagaaggca	catcgagtcc	actcgatcg	tcgggtggcag	gtaaagcggct	420
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ggagaggcc	ggagccatcc	cttcccccgg	agcccggtctc	accccccagag	tcgctcgffff	660
atggggatg	ggggatgggg	tggcatctt	tgactgtcgt	ttgctgtttt	cttctcttt	720
tgtaatagct	acagcgaaca	taattttacc	cagggttcca	ccgtggcttc	gtccgtcctc	780
ggcatctctc	agtccagtag	atacccaagg				810

Sheep PrP cDNA (SEQ ID NO:4; GenBank Accession No. AJ223072)

atggaaaa	gccacatagg	cagttggatc	ctgggtctct	ttgtggccat	gtggagtgtac	60
gtgggcctct	gcaagaageg	accaaaacct	ggcggaggat	ggaacactgg	ggggagccga	120
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gctggagcag	tggtaggggg	ccttgggtgg	tacatgtgg	gaatgtccat	gagcaggcct	420
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cccaaccaag	tgtactacag	accaggat	cagtatagta	accagaacaa	ctttgtgc	540
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ttcacccggaa	ctgacatcaa	gataatggag	cgagtgggtgg	agcaatgtg	catcaccacag	660
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cctccgtga	tcctccat	ctcttcctc	attttctca	tagtagata	ggggcaacat	780
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ccctgttagt	gtgggtgtctc	atttcttgc	tctcttgc	tacctgtata	ataataaccct	900
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ggccacataa	tattcattca	aaaaaaca	actggaaaatc	cttagtcat	agacccagg	1140
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tttgcagggt	acatttgcag	gtacacacgc	catctattgc	atcaagaatg	gatattcatg	1260
caacccctgaa	tttatggca	gaggacatct	tcacaaggaa	tgaacataat	acaaaggctt	1320
ctgagactaa	aaaattccaa	catatggaaag	aggtgc	ccctt	ggtggcagcc	1380
tatgtttaag	caccccaag	tgtatattct	ttcttttagta	acataaagta	tagataatta	1440
aggtacccat	attaaactac	ttcttagaca	ctgagagca	atctgtgtt	tatctggaaac	1500

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ccaggatgt tttgacattg cttagggatg tgagagttgg actgtaaaga aagctgagtg 1560
 ctgaagagtt catgctttt aactatagtg ttggagaaaa ctcttgagag tcccttgac 1620
 taaaaggaga tcagtcctga atattcattt gaaggactga tgctgaagct gaaactccaa 1680
 tactttggc acctgtatggg aagaactgaa ggcaggaggg atgcttagaa agactgaagg 1740
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 ccatgggtgc gcagagtccg acacgattga gtgacttaat tgaggtgacc cagatccaac 1920
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 aacttttctt gtttagagcaa ttaacatctg aaccacctaa tgcattacct gttttgtaa 2400
 ggtactttt gtaaggtaact aaggagatgt gggtttaatc cctaggcag gtaaaatcccc 2460
 tagaggaaga aatggcaacc cactccagta ttcttgccag gaaaatccag tgggcagagg 2520
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 actgaacaat aaggacaata aatgtgggtt cggctaaaag gttcattaccc ttttttctt 2640
 gtaagatggc tctagtagta cttgtcttta tcttcattcg aaacaattt gtagattgt 2700
 atgtgacagc tcttgcattca gcatgcattt gaaaaaaaaa tcacaattgg taaattttt 2760
 tatagccatc ttactattga agatgaaaga aaagaagcaa aattttcagc atatcatgct 2820
 gtacttattt caagaaagat aaccaaaatg caaaaatgtt tttgtgaagt gtatggagaa 2880
 gggctgcaa ctgatcaagc ttgtcaagttt agtttgc当地 gtttgc当地 ggagatttct 2940
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 atagaacattt gaaaaccatt tgcaccatct cagttatgtt aatcactttt atgtttgc当地 3120
 tccacataag caaaaaaaaa acaacaaaaaa aaatacaac cttgaccata ttgc当地 3180
 cagttctcta ctgaaatgtt tgaaaacact ttgtttttaa aaacagattt tgattaacag 3240
 tgggtacgat acaataacgt agatgaaaga aattgttaggg tgagatgtt gacccacacc 3300
 accaaaggcc agtcttcctc taaagaagat gtgtgtatgg tgggatttgg aagtaatcc 3360
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 ctacatattt ctttgcatttgc ccagcatggc ttggatccatctg ttttgc当地 3540
 acgttgcattt ttggattttt ttccatttat ttcaggatcttcaaaaatattca taatggaaaa 3600
 aatttccattt cccttggaaaga tgtaaagtgc atctggaaaaa ttcttgc当地 caaaaagata 3660
 aaaagttttt tgaacacaga attatgcgt tgc当地 gaaatggc当地 gtagtggaaac 3720
 aaaagatgtt ctatgttgc当地 ttggatggat tttagtgc当地 atgaaaaatg ttttgc当地 3780
 ttttgc当地 acaccaaaagg cacatttttag caacccaata ctgaatcttca agaaactct 3840
 tctgtgtgtt tgc当地 tgc当地 tagtttgc当地 aagaatccag agtgc当地 3900
 gaaatacgca tgc当地 ttttgc当地 ttgtactt gcatgtactt gttttgtttt 3960
 aaaagttttt aatattttaa tatctgacta aaattaaaca ggagctaaaa ggagctatctt 4020

Bovine PrP cDNA (SEQ ID NO:5; GenBank Accession No. X55882)

atggtaaaaa	gccacatagg	cagtggatc	ctgggttctt	tgtggccat	gtggagtgc	60
gtgggcctct	gcaagaagcg	acccaaacct	ggaggaggat	ggaacactgg	ggggagccga	120
tacccaggac	aggcgatcc	tggaggcaac	ctttatccac	ctcaggagg	gggtggctgg	180
ggtcagcccc	atggagggtgg	ctggggccag	cctcatggag	gtggctgggg	ccagcctcat	240
ggaggtggct	ggggtcagcc	ccatgggtgt	ggctggggac	agccacatgg	tgtggagggc	300
tggggtcaag	gtggtaccca	cggtaatgg	aacaaaccca	gtaagccaaa	aaccaacatg	360
aagcatgtgg	caggagctgc	tgc当地	gcagttggtag	ggggc当地	tggctacatg	420
ctgggaagtg	ccatgagcag	gccttata	cattttggca	gtgactatga	ggaccgttac	480
tatccgtggaa	acatgcaccg	ttacccaaac	caagtgtact	acaggccagt	ggatcagtat	540
agtaaccaga	acaactttgt	gcatgc当地	gtcaacatca	cagtcaagga	acacacagtc	600
accaccacca	ccaaggggaa	gaacttcacc	gaaactgaca	tcaagatgt	ggagcgagtg	660
gtggagcaaa	tgtgcattac	ccagtagccag	agagaatccc	aggcttatta	ccaaacgaggg	720
gcaagtgtga	tcctcttctc	ttcccttctt	gtgatcctcc	tcatctttt	cctcattttt	780
ctcatagtag	gatag					795

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Chicken PrP cDNA (SEQ ID NO:6; GenBank Accession No. M61145)

gaattccctc	ggcagccagc	tcctccctct	cgtatattat	tcctttctcc	ccccctacg	60
ctggatctgg	atcatctcaa	gccgagcggt	gacggctct	tggatcgctc	atacataaaat	120
atctgtgagt	cagaggaagc	aaccaccgac	cccaagacct	caccccgagc	catggctagg	180
ctcctccacca	cctgctgcct	gctggccctg	ctgctcgccg	cctgcaccga	cgtcgccctc	240
tccaagaagg	gcaaaggca	acccagtgg	gggggttggg	gcccgggag	ccatcgccag	300
cccagctacc	cccgccagcc	gggctaccct	cataaaccag	gttaccccca	taacccaggg	360
taccccccaca	accctggcta	tcccataac	cccggctacc	cccagaaccc	tggctacccc	420
cataacccag	gttacccagg	ctgggtcaa	ggctacaacc	catccagcgg	aggaagttac	480
cacaaccaga	agccatggaa	acccccc当地	accaacttca	agcacgtggc	gggggcagca	540
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tatcccaacc	gggttacta	ccgggattac	agcagccccg	tgccacagga	cgtcttcgt	720
gcccattgt	ttaacatcac	agtgcata	tacagcattt	gcccgtgtc	caagaagaac	780
acctccgagg	ctgtggcgc	agcaaacca	acggagggtgg	agatggagaa	caaagtgggt	840
acgaaggta	tccgcgagat	gtgcgtgc	cagtaccgc	agtaccgc	ggcctcgggc	900
atccagctc	accctgcata	cacctggctc	gccgtccctc	tcctccctt	caccaccctt	960
tttgcatgc	actgatggg	tgccgtgccc	cggccctgt	gcagtgagat	gacatcggt	1020
ccccgtcccc	acccatgggg	tgttcctgt	cctcgctttt	gtccatctt	ggtgaagatg	1080
tccccccgct	gcctccccgc	aggctctgt	ttgggcaaat	gggagggat	tttgcctgt	1140
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aaggagcgc	tgctaaatgc	ccaaaaaggt	aaactgt	aaaagctt	ttaatgtat	1740
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caaaaaccaa	aggatgtcaa	aatgcagcc	aaggggaaatc	aagcacgcag	gaaaggactt	1860
gaatcagctc	aactggattt	aatggcaaa	aggcatgt	agaacgaacg	gcaagggat	1920
gctggagatc	cacccctgt	gagcaattt	ttcgatgcag	ccaatgaaac	tattgttct	1980
tgtgtttag	ttgtgtgt	tgtgtacata	ggctgttagc	tatgtaaatg	tacacgtgtc	2040
aagctgtcg	cacccgtgt	agctaata	tatcatgt	gtgggcactg	aatgccaccg	2100
ttggccatac	ccaaaccgtcc	taaaacattt	tcacgtcg	gtaacttaag	tggagataca	2160
ctttcgtat	attcagcaaa	aggaattc				2188

Mouse PrP cDNA (SEQ ID NO:7; GenBank Accession No. M13685)

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cagtccaaatt	taggagagcc	aagcagacta	tcagtcatca	tggcgaacct	tggctactgg	120
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cgttaccac	ctcagggtgg	cacccctgggg	cagccccacg	gtgggtgg	gggacaaccc	300
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ctcatccctt	tgatgtgtt	atgagggagg	ccttccgt	tgttccctcg	cattctcg	900
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aggctccatt ccaaagtggg aaagaaaattc tgcttagcatt gtttaaatca ggcaaaaattt 1740
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atctgaagta tgggacgccc tggccgttcc atccagttact aaatgcttac cgtgtgaccc 1920
ttgggcttcc agcgtgcaact cagttccgta gattccaaa gcagacccct agctggtctt 1980
tgaatctgca tgtacttcaac gttttctata tttgtactt tgcatgtatt ttgtttgtc 2040
ataaaaaaag ttataaaatg tttgtatca gactgacatt aaatagaagc tatgtatg 2097

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Sheep PrP cDNA (SEQ ID NO:8; GenBank Accession No. X79912)

gcagagaagt catcatggtg aaaagccaca taggcagttt gatcctgggtt ctctttgtgg	60
ccatgtggag tgacgtgggc ctctgcaga agcgacccaa acctggcgga ggatggaaaca	120
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gaggggggtgg ctgggttcag cccatggag gtggctgggg ccaacccat ggaggtggct	240
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acaactttgt gcatgtactt gtcacacatca cagtcaagca acacacatgc accaccacca	600
ccaaggggga gaacttcacc gaaactgaca tcaagataat ggagcgagtg gtggagccaa	660
tgtcatcac ccagtaccag agagaatccc aggcttatta ccaaaggggg gcaagtgtga	720
tcctcttttc ttcccttcc tgcacccatc ctcattttt ctcatagtag	780
gatagggca accttcctgt ttt	803

Rat PrP cDNA (SEQ ID NO:9; GenBank Accession No. NM_012631)

atggcgaacc ttggctactg gctgtggcc ctctttgtga ctacatgtac tcatgtttggc	60
ctctgcaaaa agcggccaaa gcctggaggg tggaaacactg gtggaaagccg gtaccctggg	120
cagggaaagcc ctggaggca cggtaacccca cctcagatgt gtggcacctg ggggcagccc	180
catgggtgtg gctggggaca acctcatggt ggtggctggg gacaacccatca tgggtggc	240
tgggttcage cccatggcg gggctggagt caaggagggg gtaccatcaa tcagtggAAC	300
aagcccagca agccaaaaac caacctcaag catgtggcag gggctgccgc agctggggca	360
gtagtggggg gccttgggg ctacatgtt gggagtgcca tgagcaggcc catgctccat	420
tttggcaacg actggggagga cgcctactac cgagaaaaaca tgcacccatca cccatccaa	480
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aatatcacca tcaaggacgca tacagtccacc accaccacca agggggagaa cttcacggag	600
accgacgtga agatgtatgg gctgtgggt gaggcagatgt gctcacccca gatcagaag	660
gagtcccgagg cctattacga cgggagaaga tctagcggcc tgctttctc cttccctcc	720
tgatcctcc tcatctcatt cctcatcttc ctgatcgtgg gatga	765

As used herein, "prion disease" refers to any disease or condition in a subject, the pathogenesis of which involves a prion protein other than PrP^C of the species of the subject.

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A prion disease will typically but not necessarily be a transmissible spongiform encephalopathy.

As used herein, “transmissible spongiform encephalopathy” and, equivalently, “(TSE)” shall mean any prion disease that is associated with spongiform encephalopathy and is communicable from one individual to another. As prion diseases can include entities other than TSE, this term refers to at least a subset of all prion diseases. At present TSE includes Creutzfeldt-Jakob disease, kuru, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, bovine spongiform encephalopathy, and scrapie.

As used herein, “Creutzfeldt-Jakob disease” and, equivalently, “(CJD)” refers to the TSE that naturally occurs in humans. CJD includes sporadic, genetic (familial), and infectious (i.e., variant and iatrogenic) forms. CJD is a well described entity in the medical literature, and until now has been widely believed to be a uniformly fatal neurodegenerative disease for which there is no effective form of treatment.

As used herein, “variant Creutzfeldt-Jakob disease” and, equivalently, “(vCJD)”, also referred to in the literature as new variant Creutzfeldt-Jakob disease (nvCJD), refers to CJD attributable to the BSE prion. It can be distinguished from sporadic CJD not only by the prion involved but also by certain clinical and preclinical features. See Aguzzi A (2000) *Haematologica* 85:3-10; Hill AF et al. (1997) *Nature* 389:448-50; Bruce ME et al. (1997) *Nature* 389:498-501; Will R et al. (1996) *Lancet* 347:921-5.

As used herein, “iatrogenic Creutzfeldt-Jakob disease” and, equivalently, “(iCJD)” refers to any form of CJD that is attributable to work- or treatment-related exposure to prion protein that is associated with CJD.

As used herein, “bovine spongiform encephalopathy” and, equivalently, “(BSE)” shall refer to the TSE that occurs naturally in cows and cattle.

As used herein, “scrapie” refers to the TSE that occurs naturally in sheep and goats, as well as to experimental models of scrapie. Scrapie in sheep has been recognized and described in the literature for over 300 years. For a review, see O’Rourke KI (2001) *Vet Clin North Am Food Anim Pract* 17:283-300.

As used herein, a “prion protein that is associated with a prion disease” refers to any prion protein involved in the pathogenesis of a prion disease. A prion protein that is associated with a prion disease can be a prion found in nature. Alternatively, prion protein that is associated with a prion disease can be a prion protein made de novo or modified from

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its natural form through human activity, e.g., by in vitro synthesis, chemical synthesis, chemical derivatization, or genetic alteration. Chemical alteration includes, without limitation, altered glycosylation. In a preferred embodiment, a prion protein that is associated with a prion disease is a prion protein found in nature, e.g., PrP^{Sc}. In one embodiment, a prion protein that is associated with a prion disease is a truncated or genetically modified form of a prion protein found in nature. A genetically modified form of a prion protein found in nature includes a fusion protein involving at least a substantial portion of a prion protein as one component, a prion protein that differs from a prion protein found in nature by one or more conservative amino acid substitutions, and allelic variants of the prion protein found in nature.

Naturally occurring residues can be divided into the following classes based on common side chain properties: (1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr; (3) acidic: Asp, Glu; (4) basic: Asn, Gln, His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. Thus, for example, conservative amino acid substitutions can involve the exchange of a member from one of these classes for another member from the same class. Non-conservative amino acid substitutions can involve the exchange of a member of one of these classes for a member from another class.

A conservative amino acid substitution can involve a substitution of a native amino acid residue with another residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues that are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties.

As used herein, "prion protein:scrapie form (PrP^{Sc})" refers to any of a number of naturally occurring, species-specific, proteinase K-resistant forms of prion protein associated with prion disease. Specific examples include, but are not limited to, the following: human PrP^{Sc} having an amino acid sequence provided by SEQ ID NO:10; bovine PrP^{Sc} having an amino acid sequence provided by SEQ ID NO:11; bovine PrP^{Sc} having an amino acid sequence provided by SEQ ID NO:12; ovine PrP^{Sc} having an amino acid sequence provided by SEQ ID NO:13; ovine PrP^{Sc} having an amino acid sequence provided by SEQ ID NO:14; and murine PrP^{Sc} having an amino acid sequence provided by SEQ ID NO:15.

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Human PrP^{Sc} (SEQ ID NO:10; GenBank Accession No. AAE81600)

MANLGCWMLV LFVATWSDLG LCKKRKPKPG WNTGGSRYPG QGSPGGNRYP PQGGGGWGQP	60
HGGGWGQPHG CGWGQPHGGG WGQPHGGGW QGGGTHSQWN KPSKPKNM HMAAGAAAAGA	120
VVGGLGGYML GSAMSRPIIH FGSDYEDRYY RENMHRYPNQ VYYRPMDDEYS NQNNFVHDCV	180
NITIKQHTVT TTTKGENFTE TDVKMMERVV EQMCITQYER ESQAYYQRGS SMVLFSSPPV	240
ILLISFLIFL IVG	253

Bovine PrP^{Sc} (SEQ ID NO:11; GenBank Accession No. AAE81601)

MVKSHIGSWI LVLFVAMWSD VGLCKKRKPK GGWNNTGGSRY PGQGSPGGNR YPPQGGGGWG	60
QPHGGGWGQP HGGGWGQPHG CGWGQPHGGG WGQPHGGGW GQGGTHGQWN KPSKPKNM	120
HVAGAAAAGA VVGGLGGYML GSAMSRPLIH FGSDYEDRYY RENMHRYPNQ VYYRPVDQYS	180
NQNNFVHDCV NITVKEHTVT TTTKGENFTE TDVKMMERVV EQMCVTQYQK ESQAYYDQGA	240
SVILFSSPPV ILLISFLIFL IVG	263

Bovine PrP^{Sc} (SEQ ID NO:12; GenBank Accession No. CAA39368)

MVKSHIGSWI LVLFVAMWSD VGLCKKRKPK GGGWNTGGSR YPGQGSPGGN RYPPQGGGGW	60
GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GWGQPHGGGG WGQGGTHGQW NKPSKPKNM	120
KHVAGAAAAG AVVGGGLGGY LGSAMSRPLI HFQSDYEDRY YYRENMYRPN QVYYRPVDQY	180
SNQNNFVHDC VNITVKEHTV TTTTAKGENFT ETDIKMMERVV VEQMCITQYQ RESQAYYQRG	240
ASVILFSSPP VILLISFLIF LIVG	264

Ovine PrP^{Sc} (SEQ ID NO:13; GenBank Accession No. AAE81602)

MVKSHIGSWI LVLFVAMWSD VGLCKKRKPK GGWNNTGGSRY PGQGSPGGNR YPPQGGGGWG	60
QPHGGGWGQP HGGGWGQPHG GSGWQPHGGG GWGQGGSHSQ WNKPSKPKT MKHVAGAAA	120
GAVVGGGLGGY MLGSAMSRPL IHFGNDYEDR YYRENMYRYP NQVYYRPVDQ YSNQNNFVHD	180
CVNITVKQHT VTTTTKGENF TETDIKIMER VVEQMCITQY QRESQAYYQR GASVILFSSP	240
PVILLISFLI FLIVG	255

Ovine PrP^{Sc} (SEQ ID NO:14; GenBank Accession No. CAA56283)

MVKSHIGSWI LVLFVAMWSD VGLCKKRKPK GGGWNTGGSR YPGQGSPGGN RYPPQGGGGW	60
GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GWGQGGSHS QWNKPSKPKT NMKHVAGAAA	120
AGAVVGGGLGG YMLGSAMSRP LIHFNDYED RYYRENMYRYP PNQVYYRPVD QYSNQNNFVH	180
DCVNITVKQH TVTTTTKGEN FTETDIKIME RVVEQMCITQ YQRESQAYYQ RGASVILFSS ..	240
PPVILLISFL IFLIVG	256

Murine PrP^{Sc} (SEQ ID NO:15; GenBank Accession No. AAE81599)

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MANLGYWLLA	LFVTMWTDVG	LCKKRPKPGG	WNTGGSRYPG	QGSPGGNRYP	PQGGTWGQPH	60
GGGWGQPHGG	SWGQPHGGSW	GQPHGGGWGQ	GGGTHNQWNK	PSKPKTNLKH	VAGAAAAGAV	120
VGGLGYYMLG	SAMSRPMIHF	GNDWEDRYYR	ENMYRYPNQV	YYRPVDQYSN	QNNFVHDCVN	180
ITIKQHTVTT	TTKGGENFTET	DVKMMERVVE	QMCVTQYQKE	SQAYYDGRRS	SSTVLFSSPP	240
VILLISFLIF	LIVG					254

As used herein, “full-length PrP^{Sc}” refers to a form of PrP^{Sc} that includes all its amino acids as it occurs in nature. It is to be distinguished, for example, from a truncated form of PrP^{Sc}, described elsewhere herein. A full-length PrP^{Sc} can, however, be incorporated into a PrP^{Sc} conjugate or PrP^{Sc} fusion protein.

As used herein, a “derivative of PrP^{Sc}” refers to a chemical or genetic derivative of a naturally occurring form of PrP^{Sc}, including PrP^{Sc} with non-native glycosylation, covalent or non-covalent conjugates formed between PrP^{Sc} and another compound, PrP^{Sc} fusion proteins, and any combination thereof. A “derivative of a fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc}” refers to a chemical or genetic derivative of an N-terminally truncated form of PrP^{Sc}, including such truncated forms of PrP^{Sc}: (i) with non-native glycosylation, (ii) as part of a covalent or non-covalent conjugate formed with another compound, (iii) as part of a fusion protein, or (iv) any combination thereof. In preferred embodiments the fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc} refers to a fragment lacking one or more, up to and including all, of the octarepeats (e.g., GGGWGQPH (SEQ ID NO:16) and GGSWGQPH (SEQ ID NO:17)). See Flechsig E et al. (2000) *Neuron* 27:399-408.

A truncated form of a prion protein found in nature is identical in primary sequence to the prion protein found in nature except for the absence of one or more amino acid residues from the N-terminal end, the C-terminal end, or both the N-terminal and the C-terminal ends. Truncated forms can also include deletion mutants, in which an internal sequence is omitted without changing either the N-terminal end or the C-terminal end. In a preferred embodiment, a truncated prion protein lacks one or more, up to and including all, N-terminal octarepeats (e.g., GGGWGQPH and GGSWGQPH). See Flechsig E et al. (2000) *Neuron* 27:399-408.

As used herein, a “fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc}” shall refer to a truncated form of full-length PrP^{Sc} lacking one or more N-terminal amino acids normally present in full-length PrP^{Sc}. In a preferred embodiment, the fragment

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lacks one or more, up to and including all, of the octarepeats (e.g., GGGWGQPH and GGSGQPH). See Flechsig E et al. (2000) *Neuron* 27:399-408.

The methods of the instant invention employ immunostimulatory nucleic acids. In the preferred embodiment, the immunostimulatory nucleic acid is a CpG nucleic acid. The terms "nucleic acid" and "oligonucleotide" are used interchangeably to mean multiple nucleotides (i.e., molecules comprising a sugar (e.g., ribose or deoxyribose) linked to a phosphate group and to an exchangeable organic base, which is either a substituted pyrimidine (e.g., cytosine (C), thymidine (T) or uracil (U)) or a substituted purine (e.g., adenine (A) or guanine (G)). As used herein, the terms "nucleic acid" and "oligonucleotide" refer to oligoribonucleotides as well as oligodeoxyribonucleotides. The terms "nucleic acid" and "oligonucleotide" shall also include polynucleosides (i.e., a polynucleotide minus the phosphate) and any other organic base containing polymer. Nucleic acid molecules can be obtained from existing nucleic acid sources (e.g., genomic or cDNA), but are preferably synthetic (e.g., produced by nucleic acid synthesis).

The terms "nucleic acid" and "oligonucleotide" also encompass nucleic acids or oligonucleotides with substitutions or modifications, such as in the bases and/or sugars. For example, they include nucleic acids having backbone sugars that are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 2' position and other than a phosphate group at the 5' position. Thus modified nucleic acids may include a 2'-O-alkylated ribose group. In addition, modified nucleic acids may include sugars such as arabinose instead of ribose. Thus the nucleic acids may be heterogeneous in backbone composition thereby containing any possible combination of polymer units linked together such as peptide-nucleic acids (which have an amino acid backbone with nucleic acid bases).

Nucleic acids also include substituted purines and pyrimidines such as C-5 propyne modified bases. Wagner RW et al. (1996) *Nat Biotechnol* 14:840-4. Purines and pyrimidines include but are not limited to adenine, cytosine, guanine, thymidine, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, and other naturally and non-naturally occurring nucleobases, substituted and unsubstituted aromatic moieties. Other such modifications are well known to those of skill in the art.

The immunostimulatory oligonucleotides of the instant invention can encompass various chemical modifications and substitutions, in comparison to natural RNA and DNA, involving a phosphodiester internucleoside bridge, a β -D-ribose unit and/or a natural

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nucleoside base (adenine, guanine, cytosine, thymine, uracil). Examples of chemical modifications are known to the skilled person and are described, for example, in Uhlmann E et al. (1990) *Chem Rev* 90:543; "Protocols for Oligonucleotides and Analogs" Synthesis and Properties & Synthesis and Analytical Techniques, S. Agrawal, Ed, Humana Press, Totowa, USA 1993; Crooke ST et al. (1996) *Annu Rev Pharmacol Toxicol* 36:107-129; and Hunziker J et al. (1995) *Mod Synth Methods* 7:331-417. An oligonucleotide according to the invention can have one or more modifications, wherein each modification is located at a particular phosphodiester internucleoside bridge and/or at a particular β -D-ribose unit and/or at a particular natural nucleoside base position in comparison to an oligonucleotide of the same sequence which is composed of natural DNA or RNA.

For example, the invention relates to an oligonucleotide which comprises one or more modifications and wherein each modification is independently selected from:

- a) the replacement of a phosphodiester internucleoside bridge located at the 3' and/or the 5' end of a nucleoside by a modified internucleoside bridge,
- b) the replacement of phosphodiester bridge located at the 3' and/or the 5' end of a nucleoside by a dephospho bridge,
- c) the replacement of a sugar phosphate unit from the sugar phosphate backbone by another unit,
- d) the replacement of a β -D-ribose unit by a modified sugar unit, and
- e) the replacement of a natural nucleoside base by a modified nucleoside base.

More detailed examples for the chemical modification of an oligonucleotide are as follows.

A phosphodiester internucleoside bridge located at the 3' and/or the 5' end of a nucleoside can be replaced by a modified internucleoside bridge, wherein the modified internucleoside bridge is for example selected from phosphorothioate, phosphorodithioate, NR¹R²-phosphoramidate, boranophosphate, α -hydroxybenzyl phosphonate, phosphate-(C₁-C₂₁)-O-alkyl ester, phosphate-[(C₆-C₁₂)aryl-(C₁-C₂₁)-O-alkyl]ester, (C₁-C₈)alkyl-phosphonate and/or (C₆-C₁₂)-arylphosphonate bridges, (C₇-C₁₂)- α -hydroxymethyl-aryl (e.g., disclosed in WO 95/01363), wherein (C₆-C₁₂)aryl, (C₆-C₂₀)aryl and (C₆-C₁₄)aryl are optionally substituted by halogen, alkyl, alkoxy, nitro, cyano, and where R¹ and R² are, independently of each other, hydrogen, (C₁-C₁₈)-alkyl, (C₆-C₂₀)-aryl, (C₆-C₁₄)-aryl-(C₁-C₈)-alkyl, preferably hydrogen, (C₁-C₈)-alkyl, preferably (C₁-C₄)-alkyl and/or methoxyethyl, or R¹ and R² form, together with

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the nitrogen atom carrying them, a 5-6-membered heterocyclic ring which can additionally contain a further heteroatom from the group O, S and N.

The replacement of a phosphodiester bridge located at the 3' and/or the 5' end of a nucleoside by a dephospho bridge (dephospho bridges are described, for example, in Uhlmann E and Peyman A in "Methods in Molecular Biology", Vol. 20, "Protocols for Oligonucleotides and Analogs", S. Agrawal, Ed., Humana Press, Totowa 1993, Chapter 16, pp. 355ff), wherein a dephospho bridge is for example selected from the dephospho bridges formacetal, 3'-thioformacetal, methylhydroxylamine, oxime, methylenedimethyl-hydrazo, dimethylenesulfone and/or silyl groups.

A sugar phosphate unit (i.e., a β -D-ribose and phosphodiester internucleoside bridge together forming a sugar phosphate unit) from the sugar phosphate backbone (i.e., a sugar phosphate backbone is composed of sugar phosphate units) can be replaced by another unit, wherein the other unit is for example suitable to build up a "morpholino-derivative" oligomer (as described, for example, in Stirchak EP et al. (1989) *Nucleic Acids Res* 17:6129-41), that is, e.g., the replacement by a morpholino-derivative unit; or to build up a polyamide nucleic acid ("PNA"; as described for example, in Nielsen PE et al. (1994) *Bioconjug Chem* 5:3-7), that is, e.g., the replacement by a PNA backbone unit, e.g., by 2-aminoethylglycine.

A β -ribose unit or a β -D-2'-deoxyribose unit can be replaced by a modified sugar unit, wherein the modified sugar unit is for example selected from β -D-ribose, α -D-2'-deoxyribose, L-2'-deoxyribose, 2'-F-2'-deoxyribose, 2'-O-(C₁-C₆)alkyl-ribose, preferably 2'-O-(C₁-C₆)alkyl-ribose is 2'-O-methylribose, 2'-O-(C₂-C₆)alkenyl-ribose, 2'-[O-(C₁-C₆)alkyl-O-(C₁-C₆)alkyl]-ribose, 2'-NH₂-2'-deoxyribose, β -D-xylo-furanose, α -arabinofuranose, 2,4-dideoxy- β -D-erythro-hexo-pyranose, and carbocyclic (described, for example, in Froehler J (1992) *Am Chem Soc* 114:8320) and/or open-chain sugar analogs (described, for example, in Vandendriessche et al. (1993) *Tetrahedron* 49:7223) and/or bicyclosugar analogs (described, for example, in Tarkov M et al. (1993) *Helv Chim Acta* 76:481).

A natural nucleoside base can be replaced by a modified nucleoside base, wherein the modified nucleoside base is for example selected from hypoxanthine, uracil, dihydrouracil, pseudouracil, 2-thiouracil, 4-thiouracil, 5-aminouracil, 5-(C₁-C₆)-alkyluracil, 5-(C₂-C₆)-alkenyluracil, 5-(C₂-C₆)-alkynyluracil, 5-(hydroxymethyl)uracil, 5-chlorouracil, 5-fluorouracil, 5-bromouracil, 5-hydroxycytosine, 5-(C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenylcytosine, 5-(C₂-C₆)-alkynylcytosine, 5-chlorocytosine, 5-fluorocytosine,

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5-bromocytosine, N²-dimethylguanosine, 2,4-diamino-purine, 8-azapurine, a substituted 7-deazapurine, preferably 7-deaza-7-substituted and/or 7-deaza-8-substituted purine or other modifications of a natural nucleoside bases. This list is meant to be exemplary and is not to be interpreted to be limiting.

The oligonucleotides of the present invention are nucleic acids that contain specific sequences found to elicit an immune response. These specific sequences that elicit an immune response are referred to as "immunostimulatory motifs", and the oligonucleotides that contain immunostimulatory motifs are referred to as "immunostimulatory nucleic acid molecules" and, equivalently, "immunostimulatory nucleic acids" or "immunostimulatory oligonucleotides". The immunostimulatory oligonucleotides of the invention thus include at least one immunostimulatory motif.

In one embodiment of the invention the immunostimulatory oligonucleotides include immunostimulatory motifs which are "CpG dinucleotides". A CpG dinucleotide can be methylated or unmethylated. An immunostimulatory nucleic acid containing at least one unmethylated CpG dinucleotide is a nucleic acid molecule which contains an unmethylated cytosine-guanine dinucleotide sequence (i.e., an unmethylated 5' cytosine followed by 3' guanosine and linked by a phosphate bond) and which activates the immune system; such an immunostimulatory nucleic acid is a CpG nucleic acid. CpG nucleic acids have been described in a number of issued patents, published patent applications, and other publications, including U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; and 6,339,068.

An immunostimulatory nucleic acid containing at least one methylated CpG dinucleotide is a nucleic acid which contains a methylated cytosine-guanine dinucleotide sequence (i.e., a methylated 5' cytosine followed by a 3' guanosine and linked by a phosphate bond) and which activates the immune system. In other embodiments the immunostimulatory oligonucleotides are free of CpG dinucleotides. These oligonucleotides which are free of CpG dinucleotides are referred to as non-CpG oligonucleotides, and they have non-CpG immunostimulatory motifs. The invention, therefore, also encompasses nucleic acids with other types of immunostimulatory motifs, which can be methylated or unmethylated. The immunostimulatory oligonucleotides of the invention, further, can include any combination of methylated and unmethylated CpG and non-CpG

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immunostimulatory motifs. In some embodiments the immunostimulatory oligonucleotide is not an antisense oligonucleotide.

As used herein, a "Toll-like receptor (TLR) that signals in response to the CpG nucleic acid" refers to any TLR that engages or initiates an intracellular signaling pathway associated with the development of an immune response, as a result of contacting the TLR with CpG nucleic acid. The pathway typically involves the adaptor protein MyD88 and subsequent downstream molecules including TRAF, IRAK, Jun, Erk, p38 MAPK, and NF- κ B. The TLRs are a family of at least ten highly conserved receptors that share as a common feature a cytoplasmic Toll homology IL-1 receptor (TIR) domain believed to be involved in such signaling. TLR9 is reported to be the natural receptor for CpG nucleic acid.

As to CpG nucleic acids, it has recently been described that there are different classes of CpG nucleic acids. One class is potent for activating B cells but is relatively weak in inducing IFN- α and NK cell activation; this class has been termed the B class. The B class CpG nucleic acids typically are fully stabilized and include an unmethylated CpG dinucleotide within certain preferred base contexts. See, e.g., U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; and 6,339,068. Another class is potent for inducing IFN- α and NK cell activation but is relatively weak at stimulating B cells; this class has been termed the A class. The A class CpG nucleic acids typically have stabilized poly-G sequences at 5' and 3' ends and a palindromic phosphodiester CpG dinucleotide-containing sequence of at least 6 nucleotides. See, for example, published patent application PCT/US00/26527 (WO 01/22990). Yet another class of CpG nucleic acids activates B cells and NK cells and induces IFN- α ; this class has been termed the C class. The C class CpG nucleic acids typically are fully stabilized, include a B class-type sequence and a GC-rich palindrome or near-palindrome. This class has been described in published patent application PCT/US02/26468 (WO 03/015711), the entire content of which is incorporated herein by reference.

Immunostimulatory oligonucleotides are effective in vertebrates. Different immunostimulatory oligonucleotides can cause optimal immune stimulation depending on the type of subject and the sequence of the immunostimulatory oligonucleotide. Many vertebrates have been found according to the invention to be responsive to the same class of immunostimulatory oligonucleotides, sometimes referred to as human specific immunostimulatory oligonucleotides. Rodents, however, respond to different nucleic acids.

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Immunostimulatory oligonucleotides causing optimal stimulation in humans may not generally cause optimal stimulation in a mouse and vice versa. An immunostimulatory oligonucleotide causing optimal stimulation in humans often does, however, cause optimal stimulation in other animals such as cow, horses, sheep, etc. For example, within Class B CpG ODN, preferred immunostimulatory sequences have been identified for use in mice (ODN 1826, 5'- TCCATGACGTTCTGACGTT -3', SEQ ID NO:18) and for use in humans (ODN 2006, 5'- TCGTCGTTTGTGCGTTTGTCGTT -3', SEQ ID NO:19). One of skill in the art can identify the optimal immunostimulatory nucleic acid sequences useful for a particular species of interest using routine assays described herein and/or known in the art, using the guidance supplied herein.

As used herein, the term "treat" as used in reference to a disease or condition shall mean to intervene in such disease or condition so as to prevent or slow the development of, slow the progression of, halt the progression of, or eliminate the disease or condition. Thus the phrase "to treat the prion disease" as used herein means to prevent or slow the development of, slow the progression of, halt the progression of, or eliminate the prion disease.

As used herein, a "subject" refers to a human or non-human vertebrate. Preferred non-human vertebrates include feed livestock susceptible to TSE, including cows and cattle, sheep, goats, and pigs. Non-human subjects also specifically include non-human primates as well as rodents. Non-human subjects also include, without limitation, chickens, horses, dogs, cats, guinea pigs, hamsters, mink, and rabbits.

As used herein, a "subject having a prion disease" is a subject known or diagnosed to have a prion disease as disclosed herein. Generally a subject having a prion disease will have some objective manifestation of the prion disease, such as a sign, symptom, or result of a suitable diagnostic test that indicates the presence of a prion disease. In the transmissible spongiform encephalopathies, such objective manifestations can include dementia, ataxia, myoclonus, tremor, presence of protease-resistant prion protein in brain extract, and typical or characteristic abnormalities on brain CT, brain MRI, and/or EEG. This list is not meant to be limiting in any way, and those of skill in the art will recognize what criteria are suitable for making a diagnosis of prion disease in a given species in question. A subject having a prion disease shall also include any subject having a test result which specifically indicates the presence in that subject of any amount of prion protein that is associated with a prion

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disease, since it is believed that prion protein that is associated with a prion disease is not present in a subject without a prion disease.

A subject having a prion disease can but need not necessarily have an identifiable risk factor for having a prion disease. An identifiable risk factor for having a prion disease can include a family history of prion disease, a history of consuming known or suspected prion-diseased tissue, or a history of exposure to a prion protein that is associated with a prion disease or to a product derived from a known or suspected prion-diseased tissue (e.g., through administration of pituitary extract).

As used herein, a "subject at risk of developing a prion disease" is a subject with a known or suspected exposure to prion-diseased tissue, a known or suspected exposure to prion protein that is associated with a prion disease, or a known or suspected predisposition to develop a prion disease (e.g., family history of prion disease). In one embodiment the subject at risk of developing a prion disease is a subject residing in or traveling to an area in which TSE is endemic. In one embodiment the subject at risk of developing a prion disease is a subject residing in or traveling to an area in which food or water contains or is likely to contain prion protein that is associated with prion disease. In one embodiment, a subject at risk of developing a prion disease is a subject with a known or suspected iatrogenic exposure to prion-diseased tissue, e.g., neurosurgeons, neuropathologists, pathologists, nurses, morticians, histology technicians and laboratory workers at special risk of contracting iCJD. In one embodiment, a subject at risk of developing a prion disease is a subject with a known or suspected iatrogenic exposure to prion-diseased tissue through receiving a tissue or organ allograft from a subject having a prion disease. Such tissues can include, without limitation, corneas and dural grafts.

As used herein, an "effective amount" of a substance generally refers to that amount of the substance that is sufficient to bring about a desired effect. With reference to CpG nucleic acid, an "effective amount to induce an immune response to the prion protein" shall refer to that amount of CpG nucleic acid that is sufficient to induce an immune response to a particular prion protein. The immune response can occur *in vitro*, *in vivo*, *ex vivo*, and any combination thereof. An immune response to a prion protein can be measured using any suitable means to determine that an immune response occurs in association with exposure of an immune cell to the prion protein. The immune response can be antigen-specific, including any of the following: production of prion protein-specific antibody, proliferation of prion

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protein-specific lymphocytes, and cell-mediated immunity against cells expressing prion protein. The immune response can alternatively or additionally be antigen-nonspecific, including any of the following: induction of a Toll-like receptor signaling pathway, inflammation, and production of a cytokine and/or chemokine. Because prion proteins are generally believed not to evoke an immune response, any prion protein-specific immune response which occurs in association with exposure of an immune cell to a prion protein will generally indicate an immune response to the prion protein.

Also with reference to CpG nucleic acid, an "effective amount to treat the prion disease" shall refer to that amount of CpG nucleic acid that is sufficient to treat a particular prion disease. In one embodiment, an effective amount to treat the prion disease is that amount that is sufficient to slow the development of prion disease, compared to the rate of development of prion disease that would occur without CpG administration according to the instant invention. In one embodiment, an effective amount to treat the prion disease is that amount that is sufficient to prevent the development of prion disease, compared to development of prion disease that would occur without CpG administration according to the instant invention. In one embodiment an effective amount to treat the prion disease is that amount that is sufficient to slow the progression of prion disease, compared to the rate of progression of prion disease that would occur without CpG administration according to the instant invention. In one embodiment an effective amount to treat the prion disease is that amount that is sufficient to stop the progression of prion disease, compared to the progression of prion disease that would occur without CpG administration according to the instant invention. In one embodiment an effective amount to treat the prion disease is that amount that is sufficient to resolve prion disease, compared to the prion disease that would occur without CpG administration according to the instant invention.

Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular immunostimulatory oligonucleotide being administered, the antigen, the size of the subject, or the severity of the disease or condition.

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One of ordinary skill in the art can empirically determine the effective amount of a particular immunostimulatory oligonucleotide and/or other therapeutic agent without necessitating undue experimentation.

Subject doses of the immunostimulatory oligonucleotides for mucosal or local delivery typically range from about 0.1 µg to 10 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. More typically doses range from about 10 µg to 5 mg per administration, and most typically from about 100 µg to 1 mg, with repeated administrations being spaced days or weeks apart. Subject doses of immunostimulatory oligonucleotides for parenteral delivery for the purpose of inducing an antigen-specific immune response, wherein the compounds are delivered with an antigen but not another therapeutic agent are typically 5 to 10,000 times higher than the effective mucosal dose for vaccine adjuvant or immune stimulant applications, and more typically 10 to 1,000 times higher, and most typically 20 to 100 times higher. Doses of the immunostimulatory oligonucleotides for parenteral delivery for the purpose of inducing an innate immune response or for inducing an antigen-specific immune response when the immunostimulatory nucleic acids are administered in combination with other therapeutic agents or in specialized delivery vehicles typically range from about 0.1 µg to 10 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. More typically parenteral doses for these purposes range from about 10 µg to 5 mg per administration, and most typically from about 100 µg to 1 mg, with repeated administrations being spaced days or weeks apart. In some embodiments, however, parenteral doses for these purposes may be used in a range of 5 to 10,000 times higher than the typical doses described above.

For any immunostimulatory oligonucleotide the therapeutically effective amount can be initially determined from animal models. A therapeutically effective dose can also be determined from human data for CpG oligonucleotides which have been tested in humans (human clinical trials have been initiated) and for compounds which are known to exhibit similar pharmacological activities, such as other mucosal adjuvants, e.g., LT and other antigens for vaccination purposes, for mucosal or local administration. Higher doses are required for parenteral administration. The applied dose can be adjusted based on the relative bioavailability and potency of the administered immunostimulatory oligonucleotide. Adjusting the dose to achieve maximal efficacy based on the methods described herein and

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other methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan.

The immunostimulatory oligonucleotide can be administered alone or with antigen or other therapeutic agent. In this context, "antigen" refers to any biological molecule capable of eliciting specific immunity. Antigens specifically include peptides (oligopeptides, polypeptides, proteins, and glycosylated derivatives thereof), and polysaccharides. Peptide antigen can be administered preformed or as a polynucleotide encoding the peptide. Also in this context, "other therapeutic agent" includes any suitable composition useful in treating prion disease, including an antibody capable of binding a prion protein. When the immunostimulatory oligonucleotide is administered with antigen or other therapeutic agent, the immunostimulatory oligonucleotide can be administered before, concurrently with, or following administration of the antigen or other therapeutic agent. The immunostimulatory oligonucleotide and the antigen or other therapeutic agent can be formulated together or separately when the immunostimulatory oligonucleotide is administered concurrently with the antigen or other therapeutic agent. When the immunostimulatory oligonucleotide is administered before or following administration of antigen or other therapeutic agent, the immunostimulatory oligonucleotide and the antigen or other therapeutic agent can be administered by the same route of administration or by different routes of administration. In addition, when the immunostimulatory oligonucleotide is administered before or following administration of antigen or other therapeutic agent, the immunostimulatory oligonucleotide and the antigen or other therapeutic agent can be administered to the same site or to different sites.

The immunostimulatory oligonucleotides may be directly administered to the subject or may be administered in conjunction with a nucleic acid delivery complex. A nucleic acid delivery complex shall mean a nucleic acid molecule associated with (e.g., ionically or covalently bound to; or encapsulated within) a targeting means (e.g., a molecule that results in higher affinity binding to target cell (e.g., B cell surfaces and/or increased cellular uptake by target cells). Examples of nucleic acid delivery complexes include nucleic acids associated with a sterol (e.g., cholesterol), a lipid (e.g., a cationic lipid, virosome or liposome), or a target cell specific binding agent (e.g., a ligand recognized by target cell specific receptor). Preferred complexes may be sufficiently stable *in vivo* to prevent significant uncoupling prior to internalization by the target cell. However, the complex can

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be cleavable under appropriate conditions within the cell so that the nucleic acid is released in a functional form.

Delivery vehicles or delivery devices for delivering antigen and nucleic acids to surfaces have been described. The immunostimulatory oligonucleotide and/or the antigen and/or other therapeutics may be administered alone (e.g., in saline or buffer) or using any delivery vehicles known in the art. For instance the following delivery vehicles have been described: cochleates (Gould-Fogerite et al., 1994, 1996); emulsomes (Vancott et al., 1998, Lowell et al., 1997); ISCOMs (Mowat et al., 1993, Carlsson et al., 1991, Hu et., 1998, Morein et al., 1999); liposomes (Childers et al., 1999, Michalek et al., 1989, 1992, de Haan 1995a, 1995b); live bacterial vectors (e.g., *Salmonella*, *Escherichia coli*, bacillus Calmette-Guérin, *Shigella*, *Lactobacillus*) (Hone et al., 1996, Pouwels et al., 1998, Chatfield et al., 1993, Stover et al., 1991, Nugent et al., 1998); live viral vectors (e.g., Vaccinia, adenovirus, Herpes Simplex) (Gallichan et al., 1993, 1995, Moss et al., 1996, Nugent et al., 1998, Flexner et al., 1988, Morrow et al., 1999); microspheres (Gupta et al., 1998, Jones et al., 1996, Maloy et al., 1994, Moore et al., 1995, O'Hagan et al., 1994, Eldridge et al., 1989); nucleic acid vaccines (Fynan et al., 1993, Kuklin et al., 1997, Sasaki et al., 1998, Okada et al., 1997, Ishii et al., 1997); polymers (e.g., carboxymethylcellulose, chitosan) (Hamajima et al., 1998, Jabbal-Gill et al., 1998); polymer rings (Wyatt et al., 1998); proteosomes (Vancott et al., 1998, Lowell et al., 1988, 1996, 1997); sodium fluoride (Hashi et al., 1998); transgenic plants (Tacket et al., 1998, Mason et al., 1998, Haq et al., 1995); virosomes (Gluck et al., 1992, Mengiardi et al., 1995, Cryz et al., 1998); virus-like particles (Jiang et al., 1999, Leibl et al., 1998).

The immunostimulatory oligonucleotides may be administered by any means known to the skilled artisan. Routes of administration include but are not limited to oral, mucosal, parenteral, intravenous, intramuscular, intraperitoneal, intranasal, intratracheal, sublingual, subcutaneous, intradermal, inhalation, ocular, vaginal, and rectal.

The immunostimulatory oligonucleotides are administered in pharmaceutically acceptable solutions, which may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic

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gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer R (1990) *Science* 249:1527-33, which is incorporated herein by reference.

The immunostimulatory oligonucleotides and optionally other therapeutics and/or antigens may be administered *per se* (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

For oral administration, the immunostimulatory oligonucleotides can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose,

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mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

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The immunostimulatory oligonucleotides, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The immunostimulatory oligonucleotides may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the immunostimulatory oligonucleotides may also be formulated as a depot preparation. Such long acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

The term "pharmaceutically-acceptable carrier" means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a

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human or other vertebrate animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

The immunostimulatory oligonucleotides useful in the invention may be delivered in mixtures with additional adjuvant(s), other therapeutics, or antigen(s). A mixture may consist of several adjuvants in addition to the immunostimulatory oligonucleotide or several antigens or other therapeutics.

The particular mode selected will depend, of course, upon the particular adjuvants or antigen selected, the particular condition being treated and the dosage required for therapeutic efficacy. The methods of this invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of an immune response without causing clinically unacceptable adverse effects. Preferred modes of administration are discussed above.

The immunostimulatory oligonucleotides may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the immunostimulatory oligonucleotides into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the compounds into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product. Liquid dose units are vials or ampoules. Solid dose units are tablets, capsules and suppositories. For treatment of a patient, depending on activity of the compound, manner of administration, purpose of the immunization (i.e., prophylactic or therapeutic), nature and severity of the disorder, age and body weight of the patient, different doses may be necessary. The administration of a given dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units. Multiple administration of doses at specific intervals of weeks or months apart is usual for boosting the antigen-specific responses.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the compounds,

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increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di-and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the invention is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Examples

Example 1. CpG-ODN Protects Mice from Scrapie Prion.

Groups of 8 mice each were inoculated intraperitoneally with 100 µl of 10% brain homogenates from mice terminally ill with RML scrapie prion strain corresponding to an infectious challenge of approximately 10^4 LD₅₀. The mice received 0.15 pmol (30 µl of 5 nM solution) CpG-ODN (oligonucleotide 1826, 5'- TCCATGACGTTCTGACGTT -3', SEQ ID NO:18), which has been shown to be a strong inducer of innate immunity and to confer sterile immunity against certain infectious diseases. Sparwasser T et al. (2000) *Eur J Immunol* 30:3591-7. CpG-ODN was administered intraperitoneally at the time of inoculation (0h) with scrapie prions as well as 4 times at intervals of 24h (4 x q 24h); a second group received 0.15 pmol (30 µl of 5 nM solution) CpG-ODN 7h after infection (7h) and 4 times at intervals of 24h (4 x q 24h); a third group received CpG-ODN at 7h after infection and subsequently 20 times at intervals of 24h (20 x q 24h). Controls matched for age and sex were given saline instead of CpG-ODN at the identical time intervals. Additional control experiments were also performed in uninfected mice using saline and brain homogenates of

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uninfected mice. All animals were observed and scored daily for clinical signs of disease. Scrapie in mice is characterized by ataxia of gait, tremor, difficulty righting from a supine position, and tail rigidity. Occurrence of two of these four symptoms was used as the end point criterion for establishing a clinical diagnosis of scrapie. Western blots of brain homogenates were performed to confirm the diagnosis. All results were analyzed using the Student's t test (Table 1).

Table 1. Mean incubation time in C57BL/6 mice after inoculation with scrapie strain RML and treatment with CpG-ODN.

Group	Inoculation	Treatment	Regimen	Time to Terminal Disease (d) ± SD	Attack Rate
1	RML	CpG 1826	0h and 4 x q 24h	253 ± 4	8/8
2	RML	CpG 1826	7h and 4 x q 24h	250 ± 6	8/8
3	RML	CpG 1826	7h and 20 x q 24h	> 330 no disease	0/8
4	RML	Saline	0h and 4 x q 24h	183 ± 7	8/8
5	RML	Saline	7h and 4 x q 24h	181 ± 3	8/8
6	RML	Saline	7h and 20 x q 24h	181 ± 3	8/8
7	Saline	CpG 1826	0h and 4 x q 24h	No disease	0/8
8	Saline	CpG 1826	7h and 4 x q 24h	No disease	0/8
9	Saline	CpG 1826	7h and 20 x q 24h	No disease	0/8
10	Brain homogenate of uninfected mouse	CpG 1826	0h and 4 x q 24h	No disease	0/8
11	Brain homogenate of uninfected mouse	CpG 1826	7h and 4 x q 24h	No disease	0/8
12	Brain homogenate of uninfected mouse	CpG 1826	7h and 20 x q 24h	No disease	0/8

Mice infected with the RML strain which received CpG-ODN at the time of inoculation and 7h post-infection as well as 4 times at intervals of 24h showed a dramatic prolongation of survival time compared to control mice with an increase in survival time of 38% in both cases. These differences were highly significant ($p<0.0001$). The application of CpG-ODN 7h post-inoculation and 20 times at 24h intervals led to disease-free intervals of more than 330 days. All control groups which were not inoculated with the RML strain remained disease-free, and no harmful effect of CpG-ODN application was observed.

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These results showed that the application of CpG-ODN at the time of infection and 7h after infection led to a dramatic prolongation of survival time. This effect can be amplified when CpG-ODN are given for a longer period of time. The application of CpG-ODN for 20 times at 24h intervals results in a disease-free interval of >330 days, which indicates the great potential of CpG-ODN for post-exposure prophylaxis of people with exposure to infection. The mechanism of disease prevention remains to be determined, but it seems that the most likely explanation for this effect is a stimulation of TLR-expressing cells of the innate immune system, e.g., macrophages, monocytes, and especially dendritic cells. Sparwasser T et al. (2000) *Eur J Immunol* 30:3591-7. CpG-ODN has been known to induce resistance against other infectious diseases. Zimmermann S et al. (1998) *J Immunol* 160:3627-30. The induction of extreme prolongation of the incubation time or even resistance to prion disease was a surprising finding in the context of a completely different infectious agent.

The findings presented here show that administration of CpG-ODN prolongs the incubation time by 38% and may have the potential to prevent infection after repeated administration, even when high doses of infectivity are administered intraperitoneally. It may therefore be possible to prevent disease after inadvertent iatrogenic exposure with much lower infectious doses administered peripherally.

Example 2. Effect of Timing Between Exposure to Prion and Administration of CpG-ODN.

Mice are injected with RML scrapie prion or control and treated with CpG-ODN or control essentially as in Example 1, except that the interval between injection with RML scrapie prion or control and administration of CpG-ODN or control is varied. In some groups administration of CpG-ODN is delayed as much as a month following injection with RML scrapie prion or control. In some groups administration of CpG-ODN precedes injection with RML scrapie prion or control. In some groups the number and schedule of repeated administrations of CpG-ODN is varied from Example 1. Results, measured as in Example 1, show that CpG-ODN is effective even when administered more than 7h after injection with RML scrapie prion.

Example 3. Mice Protected from Scrapie Prion by CpG-ODN Develop an Immune Response to Prion.

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Mice are injected with RML scrapie prion or control and treated with CpG-ODN or control essentially as in Example 1 or Example 2. At various time points following injection with RML scrapie prion or control and treatment with CpG-ODN or control, tissue or blood samples are obtained and analyzed for prion-specific and prion-nonspecific immune response. Presence of an immune response is determined by suitable method or measurement including, without limitation, antibody titer, enzyme-linked immunosorbent assay (ELISA), flow cytometry, cell proliferation assay, cytotoxicity assay, polymerase chain reaction (PCR) and reverse transcriptase-polymerase chain reaction (RT-PCR), Western immunoblot, Northern blot, and Southern blot. General methods for these types of measurements are standard and are suitably adapted to the specific antigen or stimulus being assayed. For example, ELISA is used to measure production of various secreted products, including antibodies, cytokines and chemokines. Cytokines and chemokines in this example include interleukin (IL)-4, IL-10, IL-6, IL-12, IL-18, interferon (IFN)- α , IFN- β , IFN- γ , tumor necrosis factor (TNF), and IP-10. Flow cytometry is used to measure cell surface and intracellular proteins, including markers associated with immune cell activation. Markers associated with immune cell activation can vary with cell type but include cluster of differentiation (CD) markers such as CD86, major histocompatibility complex (MHC), inducible cytokine receptors, and certain costimulatory molecules. Results show that CpG-ODN induces an immune response to prion protein.

Example 4. Selection of CpG-ODN.

Mice are injected with RML scrapie prion or control and treated with CpG-ODN or control essentially as in Example 1 or Example 2. Various CpG-ODN are compared against ODN 1826 for their effectiveness. Results, measured as in Example 1, show that protection is related to the use of species-optimized CpG-ODN.

Example 5. Use of CpG-ODN in Alzheimer's Disease Model.

Mice genetically susceptible to developing Alzheimer's-like disease are administered CpG nucleic acid alone or CpG nucleic acid plus antigen (e.g., amyloid precursor protein or A β), either prior to or following onset of Alzheimer's-like disease. Similar mice are administered appropriate control treatment. Animals are monitored for behavioral and histologic evidence of Alzheimer's-like disease.

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The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

All references, patents and patent publications that are recited in this application are incorporated in their entirety herein by reference.

We claim:

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Claims

1. A method for treating a prion disease in a subject, comprising:
administering to a subject having or at risk of developing a prion disease a CpG nucleic acid in an effective amount to treat the prion disease.
2. The method according to claim 1, wherein the administering follows exposure of the subject to a prion protein that is associated with a prion disease.
3. The method according to claim 1, wherein the prion disease is a transmissible spongiform encephalopathy (TSE).
4. The method according to claim 1, wherein the prion disease is scrapie.
5. The method according to claim 1, wherein the prion disease is bovine spongiform encephalopathy (BSE).
6. The method according to claim 1, wherein the prion disease is variant Creutzfeldt-Jakob disease (vCJD).
7. The method according to claim 1, wherein the prion disease is iatrogenic Creutzfeldt-Jakob disease (iCJD).
8. The method according to claim 1, wherein the subject is a human.
9. A method for inducing an immune response to a prion protein, comprising:
contacting an antigen-presenting cell (APC) with a prion protein; and
contacting the APC with a CpG nucleic acid in an effective amount to induce an immune response to the prion protein.
10. The method according to claim 9, wherein the immune response is in vivo.

11. The method according to claim 9, wherein the APC is selected from the group consisting of: a B cell, a dendritic cell, a macrophage, and a monocyte.
12. The method according to claim 9, wherein the APC is a dendritic cell.
13. The method according to claim 9, wherein the APC expresses a Toll-like receptor (TLR) that signals in response to the CpG nucleic acid.
14. The method according to claim 13, wherein the TLR is TLR9.
15. The method according to claim 9, wherein the prion protein is prion protein:scrapie form (PrP^{Sc}).
16. The method according to claim 9, wherein the prion protein is a fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc} .
17. The method according to claim 9, wherein the prion protein is a derivative of PrP^{Sc} or a derivative of a fragment of PrP^{Sc} lacking at least the amino terminus of full-length PrP^{Sc} .
18. The method according to claim 9, wherein the CpG nucleic acid is a Class B CpG nucleic acid.
19. The method according to claim 9, wherein the CpG nucleic acid is a Class A CpG nucleic acid.
20. The method according to claim 9, wherein the CpG nucleic acid is a Class C CpG nucleic acid.
21. The method according to claim 9, wherein the CpG nucleic acid is optimized for use in a species of the subject.

SEQUENCE LISTING

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<211> 253
<212> PRT
<213> *Homo sapiens*

<400> 10

Met Ala Asn Leu Gly Cys Trp Met Leu Val Leu Phe Val Ala Thr Trp
 1 5 10 15

Ser Asp Leu Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn
20 25 30

Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Pro Gly Gly Asn Arg
35 40 45

Tyr Pro Pro Gln Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
 50 55 60

Trp Gly Gln Pro His Gly Gly Trp Gly Gln Pro His Gly Gly Gly
 65 70 75 80

Trp Gly Gln Pro His Gly Gly Trp Gly Gln Gly Gly Thr His
 85 90 95

Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Met
 100 105 110

Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly Tyr
 115 120 125

Met Leu Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser Asp
 130 135 140

Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln
 145 150 155 160

Val Tyr Tyr Arg Pro Met Asp Glu Tyr Ser Asn Gln Asn Asn Phe Val
 165 170 175

His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr
 180 185 190

Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg
 195 200 205

Val Val Glu Gln Met Cys Ile Thr Gln Tyr Glu Arg Glu Ser Gln Ala
 210 215 220

Tyr Tyr Gln Arg Gly Ser Ser Met Val Leu Phe Ser Ser Pro Pro Val
 225 230 235 240

Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
 245 250

<210> 11
 <211> 263
 <212> PRT
 <213> Bos taurus

<400> 11

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
1 5 10 15

Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
20 25 30

Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly
35 40 45

Asn Arg Tyr Pro Pro Gln Gly Gly Gly Trp Gly Gln Pro His Gly
50 55 60

Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly
65 70 75 80

Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly
85 90 95

Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys Pro
100 105 110

Ser Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala
115 120 125

Gly Ala Val Val Gly Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala Met
130 135 140

Ser Arg Pro Leu Ile His Phe Gly Ser Asp Tyr Glu Asp Arg Tyr Tyr
145 150 155 160

Arg Glu Asn Met His Arg Tyr Pro Asn Gln Val Tyr Tyr Arg Pro Val
165 170 175

Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His Asp Cys Val Asn Ile
180 185 190

Thr Val Lys Glu His Thr Val Thr Thr Thr Lys Gly Glu Asn Phe
195 200 205

Thr Glu Thr Asp Ile Lys Met Met Glu Arg Val Val Glu Gln Met Cys
210 215 220

Val Thr Gln Tyr Gln Lys Glu Ser Gln Ala Tyr Tyr Asp Gln Gly Ala
225 230 235 240

Ser Val Ile Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser Phe
245 250 255

Leu Ile Phe Leu Ile Val Gly
260

<210> 12
<211> 264
<212> PRT
<213> Bos taurus

<400> 12

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
1 5 10 15

Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
20 25 30

Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
35 40 45

Gly Asn Arg Tyr Pro Pro Gln Gly Gly Trp Gly Gln Pro His
50 55 60

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Trp Gly Gln Pro His
65 70 75 80

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Trp Gly Gln Pro His
85 90 95

Gly Gly Gly Trp Gly Gln Gly Thr His Gly Gln Trp Asn Lys
100 105 110

Pro Ser Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala
115 120 125

Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala
130 135 140

Met Ser Arg Pro Leu Ile His Phe Gly Ser Asp Tyr Glu Asp Arg Tyr
145 150 155 160

Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln Val Tyr Tyr Arg Pro
165 170 175

Val Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His Asp Cys Val Asn
180 185 190

Ile Thr Val Lys Glu His Thr Val Thr Thr Thr Lys Gly Glu Asn
195 200 205

Phe Thr Glu Thr Asp Ile Lys Met Met Glu Arg Val Val Glu Gln Met
210 215 220

Cys Ile Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr Gln Arg Gly
225 230 235 240

Ala Ser Val Ile Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser
245 250 255

Phe Leu Ile Phe Leu Ile Val Gly
260

<210> 13

<211> 255

<212> PRT

<213> Ovis aries

<400> 13

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
1 5 10 15

Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
20 25 30

Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly
35 40 45

Asn Arg Tyr Pro Pro Gln Gly Gly Trp Gly Gln Pro His Gly
50 55 60

Gly Gly Trp Gly Gln Pro His Gly Gly Trp Gly Gln Pro His Gly
65 70 75 80

Gly Ser Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly Gly
85 90 95

Ser His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys
100 105 110

His Val Ala Gly Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly
115 120 125

Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe Gly
130 135 140

Asn Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr Pro
145 150 155 160

Asn Gln Val Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln Asn Asn
165 170 175

Phe Val His Asp Cys Val Asn Ile Thr Val Lys Gln His Thr Val Thr
180 185 190

Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys Ile Met
195 200 205

Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln Tyr Gln Arg Glu Ser
210 215 220

Gln Ala Tyr Tyr Gln Arg Gly Ala Ser Val Ile Leu Phe Ser Ser Pro
225 230 235 240

Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
245 250 255

<210> 14

<211> 256

<212> PRT

<213> Ovis aries

<400> 14

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
1 5 10 15

Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
20 25 30

Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
35 40 45

Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Trp Gly Gln Pro His
50 55 60

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
65 70 75 80

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly
85 90 95

Gly Ser His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met
100 105 110

Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu
115 120 125

Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe
130 135 140

Gly Asn Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr
145 150 155 160

Pro Asn Gln Val Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln Asn
165 170 175

Asn Phe Val His Asp Cys Val Asn Ile Thr Val Lys Gln His Thr Val
180 185 190

Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys Ile
195 200 205

Met Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln Tyr Gln Arg Glu
210 215 220

Ser Gln Ala Tyr Tyr Gln Arg Gly Ala Ser Val Ile Leu Phe Ser Ser
225 230 235 240

Pro Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
245 250 255

<210> 15
<211> 254
<212> PRT
<213> Mus musculus

<400> 15

Met Ala Asn Leu Gly Tyr Trp Leu Leu Ala Leu Phe Val Thr Met Trp
1 5 10 15

Thr Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn
20 25 30

Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg
35 40 45

Tyr Pro Pro Gln Gly Gly Thr Trp Gly Gln Pro His Gly Gly Gly Trp
50 55 60

Gly Gln Pro His Gly Gly Ser Trp Gly Gln Pro His Gly Gly Ser Trp
65 70 75 80

Gly Gln Pro His Gly Gly Trp Gly Gln Gly Gly Gly Thr His Asn
85 90 95

Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Leu Lys His Val Ala
100 105 110

Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr Met
115 120 125

Leu Gly Ser Ala Met Ser Arg Pro Met Ile His Phe Gly Asn Asp Trp
130 135 140

Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr Pro Asn Gln Val
145 150 155 160

Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His
165 170 175

Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr
180 185 190

Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg Val
195 200 205

Val Glu Gln Met Cys Val Thr Gln Tyr Gln Lys Glu Ser Gln Ala Tyr
210 215 220

Tyr Asp Gly Arg Arg Ser Ser Ser Thr Val Leu Phe Ser Ser Pro Pro
225 230 235 240

Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
245 250

<210> 16
<211> 8
<212> PRT
<213> Mus musculus

<400> 16

Gly Gly Gly Trp Gly Gln Pro His
1 5

<210> 17
<211> 8
<212> PRT
<213> Mus musculus

<400> 17

Gly Gly Ser Trp Gly Gln Pro His
1 5

<210> 18
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 18
tccatgacgt tcctgacgtt

20

<210> 19
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 19
tcgtcgttt gtcgttttgt cgtt

24

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